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Variation in Head Length of Spermatozoa in Seven Additional Species of Insects

CHARLES ZELENY AND C. T. SENAY

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VARIATION IN HEAD LENGTH OF SPERMATOZOA IN SEVEN ADDITIONAL SPECIES OF INSECTS[1]

CHARLES ZELENY and C. T. SENAY

EIGHT FIGURES

A study of variation in head length among spermatozoa from single testes in fifteen species of animals from widely separated groups was made by the senior author and E. C. Faust ('15 a and b). The frequency distribution of the size groups in a great majority of the cases showed bimodality of such a character as to make it highly probable that in these species the spermatozoa are dimorphic as regards size. Furthermore it was shown to be highly probable that this dimorphism is the result of chromosomal differences. There is a close agreement between the ratio in the two groups as calculated on this hypothesis and the actual ratio determined by measurement.

These chromosomal differences as has now been abundantly demonstrated are related to sex determination and the size groups must be similarly related, fertilization of the eggs by spermatozoa of the upper group yielding females and by those of the lower group, males. A probability is thus opened for controlling sex as soon as living spermatozoa of the two sizes can be experimentally separated.

In view of the importance of the question of existence of two size groups and in further preparation for the experimental tests, measurements were made for seven additional species and these are described in the present paper.

Together with those formerly described twenty-two species are now available for drawing a general conclusion. This number includes all that have been measured, with two exceptions. These two are Helodrilus, a hermaphroditic form, and the

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[1] Contribution from the Zoological Laboratory of the University of Illinois No. 49.

Plymouth Rock Fowl concerning which there is a controversy on the cytological side. They are not included because they have a special interest not connected with our main hypothesis and because it is desirable to make at least one more series of measurements in each species before publishing the data. Emphasis is laid on the fact that all sets of measurements are published, because bimodal distributions may appear occasionally as a matter of chance in a uniform population. The frequency of their occurrence is therefore all-important.

The details of preparation of material and the method of measurement are in all respects similar to those described by Zeleny and Faust ('15 a) except that fixation was in all cases in osmic fumes and staining in haematoxylin. The authors are indebted to Mr. C. A. Hart for identification of the species.

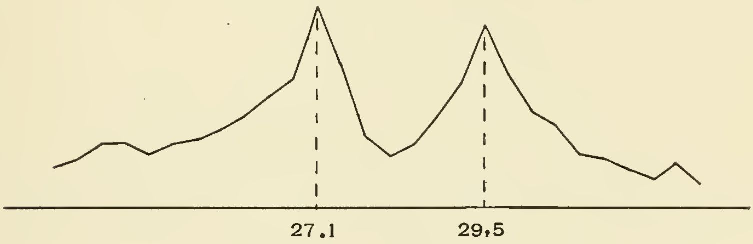
DATA

1. *Corizus lateralus,* a hemipteran. The material was obtained at the end of March and gave an abundance of active spermatozoa. Two sets of measurements, each of 500 spermatozoa, were made. As shown in figures 1 and 2 the two determinations agree closely. Each shows a pronounced bimodal curve with modes at 27.1 μ and 29.5 μ, giving a ratio of 1.00 : 1.09. The intermodal depression is deep and wide and the two elements of the curve are approximately equal as regards number of individuals. There seems to be no doubt of the existence of two distinct size groups with equal numbers of spermatozoa.

The chromosomal history was worked out by Montgomery ('06). He describes two kinds of spermatids, one with six and the other with seven chromosomes. Two size groups are therefore to be expected but the drawings given by Montgomery are not large enough to enable one to make a calculation of the chromatin ratio. In Anasa tristis, another member of the Family Coreidae, the expected ratio is 1.00 : 1.11.

2. *Leptocoris trivittatus,* a hemipteran. The material was obtained in early March and gave an abundance of active spermatozoa. Nine hundred and eighty-four measurements were made. The frequency distribution as shown in figure 3 is somewhat irregular but there are two principal modes, one at 25.4 μ

and the other at 27.8 μ, giving a ratio of 1.00 : 1.09. The majority of the individuals are grouped around the higher mode. If these modes can be considered as related to the sex chromosomes the



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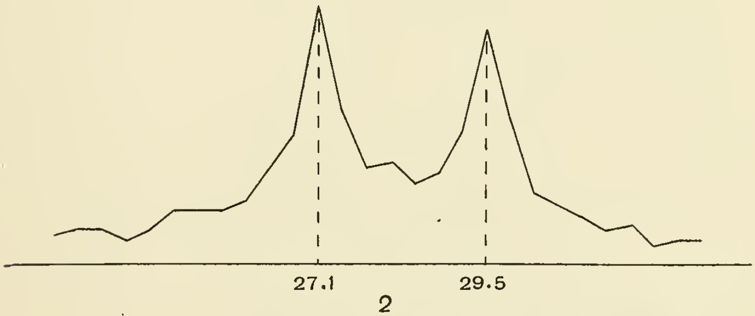

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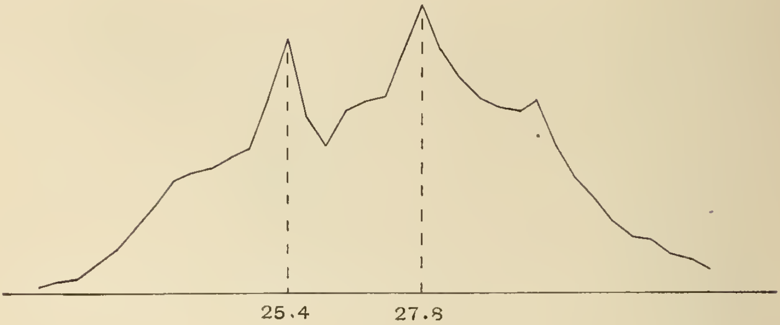
Fig. 1 Corizus lateralus; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in	23.0	23.3	23.7	24.0	24.4	24.7	25.0	25.4	25.7	26.1
Frequency	7	8	10	13	13	11	13	14	16	19
	26.4	26.7	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5
	23	27	42	30	15	11	13	19	26	38
	29.8	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6	
	28	20	17	11	10	8	6	9	5	

Fig. 2 Corizus lateralus; frequency distribution of head-lengths of another sample of 500 spermatozoa from the same testis as those given in figure 1.

Value in	23.0	23.3	23.7	24.0	24.4	24.7	25.0	25.4	25.7	26.1
Frequency	4	6	7	7	5	7	11	11	11	13
	26.4	26.7	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5
	20	27	54	32	20	21	17	19	28	45
	29.8	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6	
	30	15	12	10	7	8	4	5	5	



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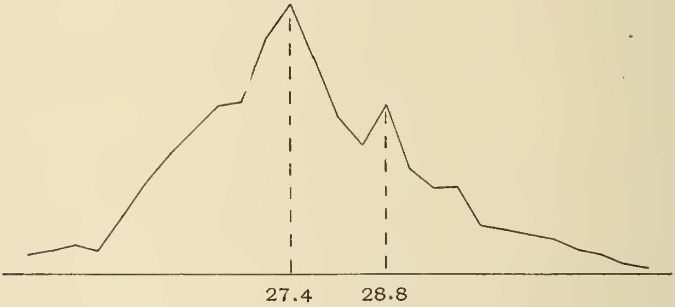

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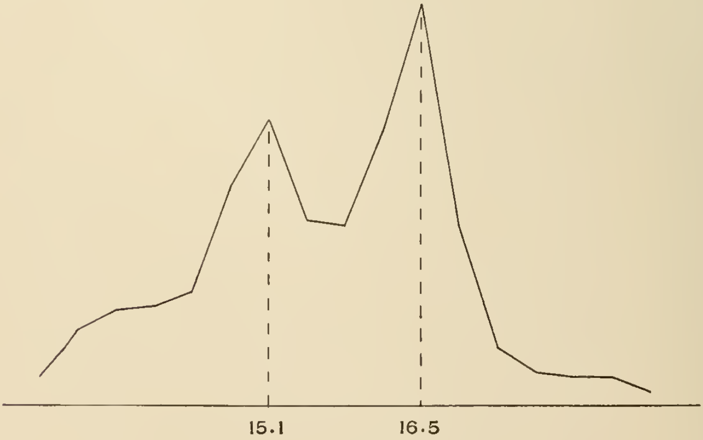

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group containing the female-determining spermatozoa exceeds the other in numbers. On the basis of the cytological evidence we should expect the two groups to be equal but there is a great deal of evidence in both cytological and experimental data to indicate that one kind is often more numerous than the other.

E. B. Wilson ('06) has described two kinds of spermatids for this species, one containing six and the other seven chromosomes. In the absence of figures it is not possible to determine an expected ratio. Since this species also is a Coreid, the ratio of 1.00 : 1.11 determined from the chromosomes of Anasa is of interest. It agrees fairly closely with the measurements of head-lengths in this as well as in the last species.

3. *Reduviolus ferus*, a hemipteran. The material was collected during March and gave active spermatozoa. The results of the five hundred measurements are given in figure 4. The fre-

Fig. 3 Leptocoris trivittatus; frequency distribution of head-lengths of 984 spermatozoa from a single testis.

Value in	20.9	21.3	21.6	22.0	22.3	22.7	23.0	23.3	23.7
Frequency	1	2	3	6	9	13	18	23	25
	24.0	24.4	24.7	25.0	25.4	25.7	26.1	26.4	26.7
	26	28	30	41	53	37	31	38	40
	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5	29.8
	41	50	60	51	45	41	39	38	40
	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6	33.0
	31	24	20	15	12	11	8	7	5

Fig. 4 Reduviolus ferus; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in	23.7	24.0	24.4	24.7	25.0	25.4	25.7	26.1	26.4
Frequency	4	5	6	5	12	19	25	30	35
	26.7	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5
	36	49	56	45	33	27	35	22	18
	29.8	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6
	18	10	9	8	7	5	4	2	1

Fig. 5 Euschistus variolarius; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in microns	13.0	13.4	13.7	14.1	14.4	14.7	15.1	15.5	15.8
Frequency	6	16	20	21	24	46	60	39	38
	16.1	16.5	16.8	17.2	17.5	17.9	18.2	18.5	
	58	84	38	12	7	6	6	3	

quency distribution gives a major mode at 27.4 μ and a minor one at 28.8 μ. The minor mode may be accidental though such an irregularity is not common in frequency distributions in a population known to be homogeneous.

There are no data available concerning the spermatogenesis of this species but Montgomery ('06) has described two kinds of spermatids for other members of the family.

4. *Euschistus variolarius,* a hemipteran. Material was obtained during April. All the spermatozoa were not fully developed and selection was necessary to insure the exclusion of the unripe ones. Five hundred measurements were made. The resulting curve as shown in figure 5 is distinctly bimodal with modes at 15.1 μ and 16.5 μ and with an inequality favoring the larger spermatozoa. The ratio between the modes is 1.00 : 1.09.

E. B. Wilson ('06) described an "X" and a "Y" chromosome for this species. The expected ratio as approximated from his figures of the chromosomes is 1.00 : 1.04. This does not agree at all well with the ratio between the modes as given above. This result is contrary to that obtained for several species by Zeleny and Faust ('15) which showed a striking similarity between the two calculations.

5. *Cosmopepla carnifex,* a hemipteran. The material was obtained in May and the spermatozoa were all active. Five hundred measurements were made. The resulting curve as given in figure 6 shows well marked bimodality with approximate equality in the two groups. The modes are at 18.5 μ and 19.9 μ and give a ratio of 1.00 : 1.075. There can be no doubt in this case of the existence of two fairly equal size groups.

Montgomery ('06) describes two kinds of spermatids for this species, one with seven chromosomes plus an "X" and the other with seven plus a "Y." The figures are not suitable for the determination of the chromatin ratio. The ratio in another Pentatomid, Euschistus variolarius, is 1.00 : 1.04.

6. *Passalus cornutus,* a coleopteran. Material was obtained at the end of March and the spermatozoa were uniformly ripe and active. Five hundred spermatozoa were measured. Neglecting the minor projection at the right, the curve as given in figure

7 is unimodal with the mode at 11.7 μ. This indicates either a single group or two so close together as to give a unimodal result (see Zeleny and Faust '15 a, p. 193).

There are no published spermatogenesis data for this species. Descriptions for different species of beetles show in all cases, except one, two groups as regards chromatin content. Leaving out the possibilities of random sampling and of selective elimination within the two groups the present species seems to be either without distinction among its spermatozoa or else has two groups differing but slightly from each other.

7. *Berosus striatus*, a coleopteran. The material was obtained in early April and the spermatozoa were all ripe and active. The frequency distribution of the five hundred measurements is given in figure 8. The curve is distinctly bimodal with approximate equality in the two groups. The modes are at 16.1 μ and 17.2 μ with a ratio of 1.00 : 1.07.

There are no cytological data for this species but as was stated in discussing the last form the descriptions for all but one species of beetles give two kinds of chromosome groups among the spermatids.

DISCUSSION

The present data as a whole substantiate the view that dimorphism in size of spermatozoa is of common occurrence in those groups of animals in which two chromosomal classes of spermatids are of common occurrence. Of the seven species described five are hemipterans and two coleopterans. In both of these orders a great majority of the species so far studied show a quantitative difference in chromosomal content among the spermatids though in each case there are some species which show no difference or only a slight one.

Among the five hemipterans three, Corizus lateralus, Euschistus variolarius and Cosmopepla carnifex, show two well marked size groups. The other two species are not so clear. One, Leptocoris trivittatus, is probably dimorphic though the curve is irregular. The other, Reduviolus ferus, is doubtful because the upper mode is very low and may be a chance projection in a unimodal distribution. It should not, however, be forgotten that the two elements of a population will, when combined, give a unimodal curve in case the modes of the elements are close together.

Among the coleopterans one, Berosus striatus, has two well marked and equal size groups. The other, Passalus cornutus, has a single size group or two with modes very close together.

Taking all cases so far described there are twenty-three species involved, including the one given by Wodsedalek ('13). This is a sufficient sample to justify us in stating that there can be no doubt of the validity of the hypothesis presented. The chromosomal dimorphism of spermatogenesis is represented in the active functional spermatozoa by size dimorphism. Control of sex then merely awaits our ability to separate the two sizes in the living condition and to use them in artificial insemination.

Fig. 6 Cosmopepla carnifex; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in microns	16.5	16.8	17.2	17.5	17.9	18.2	18.5	18.9	19.2
Frequency	6	14	16	19	26	42	83	53	35

Value in microns	19.6	19.9	20.3	20.6	20.9	21.3	21.6	22.0
Frequency	42	68	37	14	11	11	9	8

Fig. 7 Passalus cornutus; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in microns	10.15	10.30	10.45	10.60	10.80	11.00	11.15
Frequency	1	5	4	9	14	26	27

Value in microns	11.30	11.50	11.70	11.85	12.00	12.20	12.40
Frequency	46	65	89	65	55	22	29

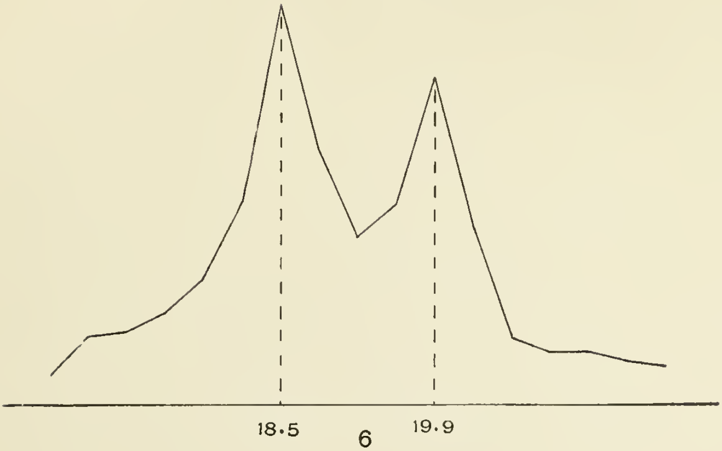
Value in microns	12.55	12.70	12.85	13.00	13.20
Frequency	14	12	7	7	2

Fig. 8 Berosus striatus; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in microns	13.7	14.1	14.4	14.7	15.1	15.5	15.8
Frequency	4	9	9	10	14	32	41

Value in microns	16.1	16.5	16.8	17.2	17.5	17.9	18.2
Frequency	56	42	30	54	40	36	30

Value in microns	18.5	18.9	19.2	19.6	19.9	20.3	20.6
Frequency	23	17	16	14	10	4	2



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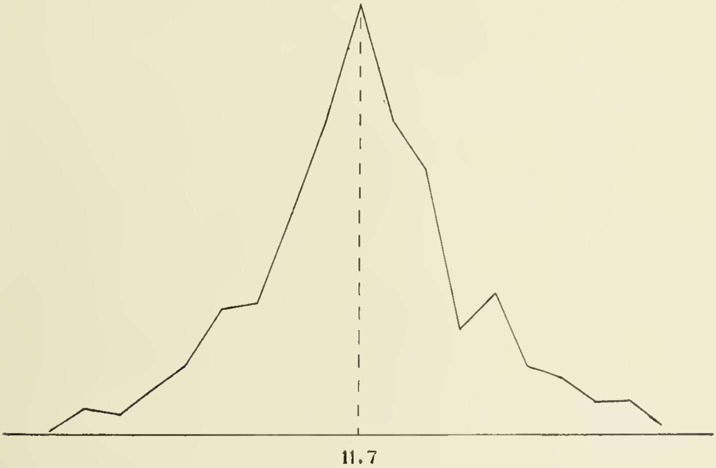

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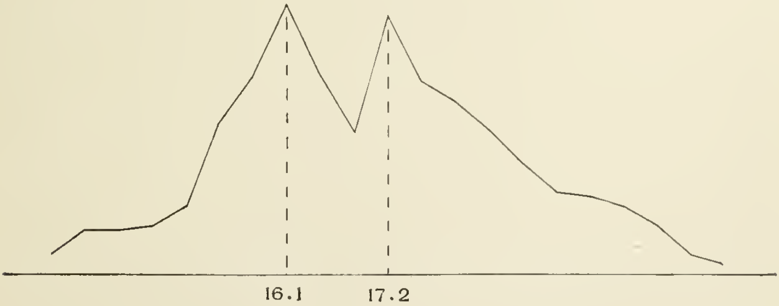

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NUCLEAR CHANGES IN THE REGENERATING SPINAL CORD OF THE TADPOLE OF RANA CLAMITANS.

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GEORGE FRED SUTHERLAND.

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NUCLEAR CHANGES IN THE REGENERATING SPINAL CORD OF THE TADPOLE OF RANA CLAMITANS.[1]

GEORGE FRED SUTHERLAND.

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I. Statement of the Problem.

The present paper gives the results of an histological study of the early stages of regeneration in the spinal cord of the frog tadpole, *Rana clamitans*. It deals especially with the degenerative nuclear changes immediately following the operation, and the phenomena of nuclear division in the formation of the new organ.

Fraisse (1885) studied these stages in several vertebrates in order to discover the origin of the regenerated tissues, and presented the following conclusions which may be used as a basis for a further detailed study.

"1. Sowohl bei Amphibien wie bei Reptilien sind verletzte Gewebe nur im Stande, wiederum gleichartig Gewebe zu erzeugen. Die Leukocyten übernehmen bei der Gewebsbildung nur die

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[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 37.

Function der Ernährung; ausserdem nehmen sie zerfallende Gewebsproducte auf und assimiliren dieselben, um sie an anderen Orten wieder zu deponiren. Niemals werden sie selbst zu fixen Gewebszellen, weder in der Bindesubstanz noch sonst wo.

"2. Sämmtliche der in Frage kommenden Gewebe der Amphibien und Reptilien sind im Stande, sich zu regeneriren; entweder direct aus ihren Elementen, oder aus einer Matrix, so lange diese Matrix unverletzt ist. Als Matrix für die Epidermis ist das Rete Malpighii, für das centrale Nervensystem das Epithel des Centralcanales, für die Muskulatur die Muskelkörperchen zu betrachten.

"3. Zuerst regeneriren sich Epithel und Bindegewebe; beides scharf getrennt, ursprünglich aus gleichartigen Zellen bestehend, die sich später differenziren."

There remains the further problem of the stages in the process by which the old organs at the cut surface replace their lost parts. Two distinct kinds of changes take place in this process, (1) degenerative and (2) regenerative. First the injured cells at the cut edge degenerate. Then follows regeneration proper, or the formation of the new organ from the remaining elements of the old.

There are three ways in which regeneration proper might take place. (1) The cells at the cut edge of each organ by dividing might extend outward, and in time form the completed organ: (2) the cells in front of the cut edge might wander backward; and (3) the cells in front of the cut edge might divide in situ and push backward the more distal cells. These possible methods of regeneration will be made clearer by a diagram of that part of the hollow neural tube extending forward from the cut (Fig. 1). If (1) (division of cells at the cut edge) were the method of regeneration, we should find after the operation that

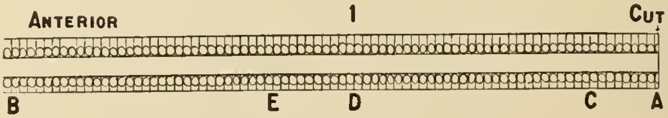

FIG. 1. Diagram, explained in the text.

the cells at the cut surface *A*, or from *A* to *C*, are dividing rapidly while from *C* to *B* about the normal number of cells is dividing.

If (2) (migration of cells) were the method, we might find no dividing cells at all, but should expect to find that the cells from *B* to *A* or possibly only from *D* to *A* are turned with their long axes parallel to the longitudinal axis of the spinal cord as if moving toward the cut end. If (3) (division of more anterior cells in situ) were the method, we should expect to find dividing cells all the way from *B* to *C* or possibly concentrated in a growing zone *ED*.

The present paper aims to give an account of the nuclear changes, both degenerative and regenerative, involved in the formation of the regenerated spinal cord.

II. MATERIAL AND METHODS.

Serial sections were made of tadpole tails killed after various regeneration periods. This enables one to follow the process from stage to stage. But to get uniform results from this method and eliminate individual variations, one must take tadpoles as nearly alike as possible at the start, operate on all at the same time, keep them under uniform laboratory conditions and make sections of several individuals at each stage.

On October 12, 1913, seventy tadpoles of *Rana clamitans*, varying in length from 30 to 60 mm., were brought into the laboratory. Two days later they were put into individual finger bowls, and forty-four medium sized individuals (32–40 mm. in length), chosen to constitute the main series, were grouped by twos or threes. Those of each group were as nearly alike as possible and each group was treated as a unit in the time of operation, killing, etc. The finger bowls were placed side by side on a table some distance from the windows so that uniform conditions of temperature, light, etc., were insured. None of the tadpoles was fed during the course of the experiment, and none died from the effects of laboratory conditions.

On October 15, the first operations were performed. Each tadpole was transferred from the finger bowl to a paraffin block and approximately one fourth of the tail was removed, with a sharp scalpel, at right angles to the plane of the tail. The animal was returned to the finger bowl and the removed part put into Gilson's killing fluid. At the end of the period of regeneration, the animals were again taken out onto the block and the regenerated tail plus a second fourth of the normal tail was removed and put immediately into Gilson's killing fluid. The times of killing were as follows: normal, immediately after the operation, 1, 3, 5½, 9½, and 14 hours, and 1, 2, 3, 4, 6, 8, 9, 10, 12, 14 and 16 days after the operation. Usual methods of technique were followed. Delafield's hæmatoxylin and acid fuchsin stain the nuclei blue and the cytoplasm pink, but do not distinctly bring out cell boundaries. For the most part sections were made in the sagittal plane.

III. Observations.

The study was confined to the histology of regeneration in the spinal cord, since a preliminary examination showed that this organ of all those in the tail was best adapted for a study of the present problem. Fig. 2 shows by a sagittal section the spinal cord, and its relation to the surrounding tissues. Fig. 3 shows a transverse section of the spinal cord alone. It is a hollow tube which distally is formed of a single layer of cells. The nuclei are very near the inner border of the cells so that there is a wide outer zone of cytoplasm but practically no inner cytoplasmic

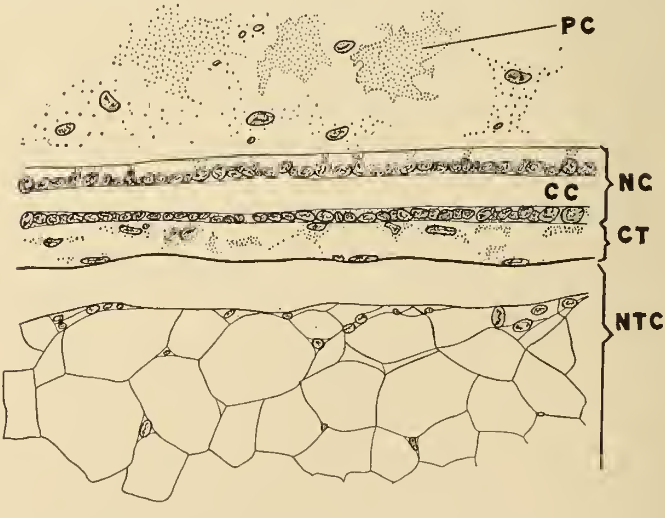

Fig. 2. Sagittal section through a part of the normal tail, showing the spinal cord and its relation to the surrounding tissues. *nc*, spinal cord; *cc*, central canal; *ntc*, notocord; *ct*, connective tissue; *pc*, pigment cell. (330 diameters.)

zone. At this stage in the development of the tadpole, the cells near the distal end of the spinal cord show little differentiation.

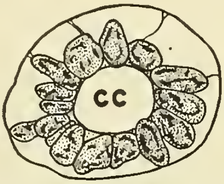

FIG. 3. Transverse section through the normal spinal cord, showing the nuclei and the outer cytoplasmic zone. *cc*, central canal. (890 diameters.)

1. *Degenerative Changes after an Operation.*

When a tadpole's tail is removed the old notocord extends out beyond the other tissues, and the connective tissue between the notocord and spinal cord is usually broken so that the spinal cord bends dorsally as in Figs. 7 and 8. A transverse cut through the tail leaves the various organs at the cut surface in contact with the surrounding medium, the water in which the tadpole lives. Sections of tadpoles killed immediately after the operation show the direct effect of the cutting (Figs. 4 and 5). Many nuclei and cells are broken and irregular in appearance and may be loosened or torn apart from each other. The injured nuclei at the cut edge and extending forward with decreasing frequency, are homogeneous in appearance and take a deep haematoxylin stain. Undoubtedly some of the nuclei are cut, and this accounts for the irregularity in shape of a good many. But a good many others, also staining deeply, are rounded and smaller than normal nuclei. These may be either normal nuclei which under the

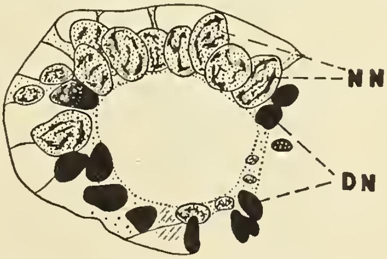

FIG. 4. Transverse section through the end of the spinal cord immediately after the operation, showing deeply-staining nuclei. *cc*, central canal; *nn*, normal nuclei; *dn*, deeply-staining nuclei. (920 diameters.)

stimulus of the operation are contracted or compressed, or cut nuclei which have rounded off. These deeply-staining nuclei, whether rounded or irregular in shape, are smaller than normal nuclei, so it may be that the chromatin, which stains deeply, is condensed on account of the loss of achromatic material.

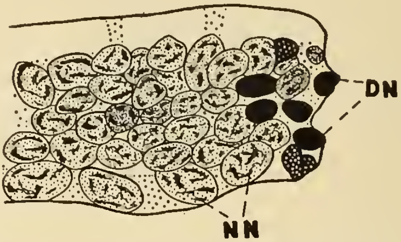

Fig. 5. Sagittal section through the side of the spinal cord immediately after the operation, showing the deeply-staining nuclei at the cut end. *dn*, deeply-staining nuclei. (920 diameters.)

The same assumption is borne out by the somewhat different appearance of nuclei in the tadpoles killed one hour after the operation (Fig. 6). Some are rounded as before; others are angular or slightly hour-glass shaped, with rather dense cytoplasm extending out from the corners. If parts of the nuclear membrane were held by the cytoplasm while the nucleus as a whole decreases in volume either by contraction or loss of achromatin, the nuclei might present such an appearance. Moreover there are gradations from hour-glass-shaped to normal nuclei

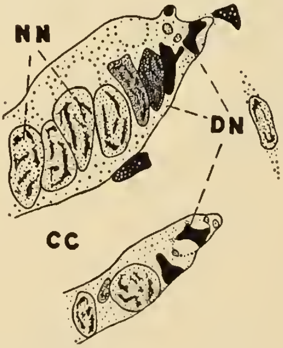

Fig. 6. Sagittal section through the spinal cord one hour after the operation. This shows the "contracting" nuclei. *cc*, central canal; *dn*, deeply-staining nuclei; *nn*, normal nuclei. (920 diameters.)

and corresponding gradations in size and depth of stain. In cases of this sort there are often vacuoles or cytoplasm between the nuclei as if the latter had shrunken, whereas in the normal cord, the nuclei are so close together that no cytoplasm can be seen between them. These facts indicate that normal nuclei become deeply staining nuclei by contraction or by loss of achromatic material.

This "contraction" of nuclei seems to be caused by contact with the water or killing fluid, or the succession of the two, as well as by direct injury from the scalpel, for other nuclei which are in contact with the exterior only through the central canal show this phenomenon. In some cases, the end of a nucleus nearest the central canal is deeply stained and contracted while the other part is normal (Fig. 4). The question immediately arises, why does not the water or other external factor enter the open neural tube and cause the contraction of the inner parts of practically all nuclei in the spinal cord? It is probably because of the presence in the tube of some substance which prevents the ready admission of external fluids, though capillarity would have a similar effect. Since the sections show very little structure within the central canal, this content must be liquid or semi-liquid. However, in a number of sections there is a rather long narrow band of cytoplasmic material which may be the more solid part of a semi-liquid substance coagulated by the killing reagent. There are other evidences of the presence of such a liquid. The sections from two of the tadpoles killed one hour after the operation show a coagulation of the outer surface of the blood plasma covering the wound, but over the spinal cord this coagulating process is delayed. The most plausible explanation seems to be that some cerebro-spinal fluid (compared by Barfurth to the cerebrospinal fluid of mammals) exerts an outward pressure which breaks through any slight hardening of the plasma at this point. Perhaps transference of the animal to a medium of different density, the killing fluid, aids the outburst. Sections of another tadpole killed at one hour show the presence of this coagulated plasma over the end of the spinal cord as well as over other parts of the tail.

The outward pressure of a fluid would tend to push out into the blood plasma any free elements such as the injured and degenerating nuclei with very little cytoplasm and hence little connection with other cells; and when this fluid breaks through, some of these nuclei may break off and float away. At one hour after the operation, broken and small rounded nuclei are seen in the end of the spinal cord and extending out into the hardened layer of the plasma, giving evidence of some force acting outward at this time (Fig. 7). Other evidences will be mentioned in describing the stages at which they appear.

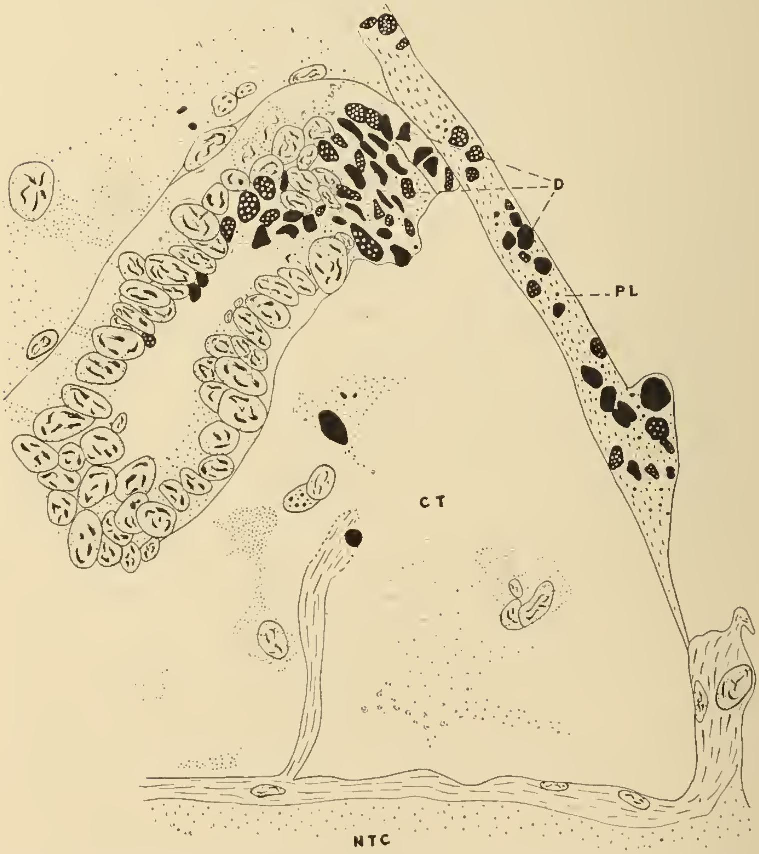

FIG. 7. Sagittal section through the spinal cord and the surrounding region one hour after the operation, showing irregularly shaped, deeply-staining nuclei in the end of the spinal cord and in the coagulated plasma layer. *dn*, deeply-staining nuclei; *pl*, plasma layer; *ct*, connective tissue; *ntc*, notocord. (1,100 diameters.)

Three hours after the operation there are fewer of the angular nuclei than at one hour and more of the round deeply-staining nuclei. The latter vary from the size of similar ones in the earlier stages down to fragments. Moreover some of the larger of these seem to be in the process of fragmentation, that is, appearances indicating stages in direct division are seen. The gradation in size and depth of stain at one hour from normal nuclei nearly to rounded ones, and the gradation down to fragments at three hours, as well as the appearances of fragmentation, make it fairly clear that normal nuclei just in front of the cut edge may contract, become rounded, and fragment. This must be a degenerative process. Even finer intermediate steps are seen in preparations of later stages.

Sections of one individual at this period appear very much like those immediately after the operation. The deeply-stained nuclei are similar, and the spinal cord is not covered either by epidermis or plasma, so that a recent outbreak of the cerebrospinal fluid must have taken place. In this case a second contact with the exterior has again started the degenerative process.

At five and a half hours the spinal cord is entirely covered by the thickened plasma layer, in which is a group of fragmenting globular nuclei. In one preparation at this time, the epidermis has closed-in over the entire wound, and there is a series of stages in the degeneration of nuclei. Some are only slightly smaller and darker than normal nuclei; others have the angular appearance characteristic of nuclei one hour after the operation, while still others are round and fragmenting. At this stage there is another evidence of the presence of a cerebrospinal fluid. The plasma covering the end of the spinal cord is pushed outward, making a knob-like extension of the central canal similar to that shown in Fig. 8. This did not appear in earlier stages either because not enough cerebrospinal fluid was present, or because the plasma layer had not coagulated sufficiently to resist the outward pressure of this fluid.

Of the two preparations of tadpoles killed after a nine and a half hour interval, one shows the epidermis and plasma covering all the wound except the neural tube; the other shows this part also covered. In the former, the sides of the neural tube are separated as if by a recent outburst of cerebrospinal fluid, and deeply-staining rounded and fragmenting nuclei are seen. In the second preparation, the deeply-staining nuclei are all small and fragmentary. In other words no more nuclei seem to be starting to degenerate.

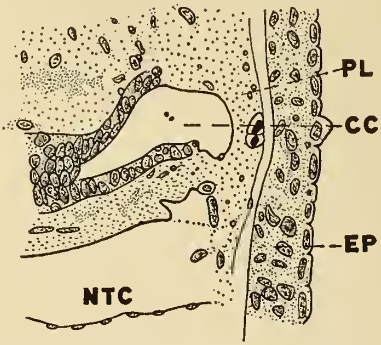

FIG. 8. Sagittal section through the end oi the spinal cord fourteen hours after the operation. This shows the epidermal layer, the plasma layer, and the knob-like extension of the central canal, caused by the outward pressure of the cerebrospinal fluid. *ep*, epidermis; *cc*, central canal; *pl*, plasma layer; *ntc*, notocord. (330 diameters.)

At fourteen hours, plasma and epidermis cover the spinal cord though the plasma is pushed outward by the cerebrospinal fluid (Fig. 8). There are nuclear fragments in the cord and degenerating nuclei in the plasma. Another preparation of the same period shows the nerve cord still open to the exterior, as well as the nuclear appearance of an earlier stage.

At twenty-four hours, only a few of the nuclei are slightly smaller and darker than the normal. At this time there appear near the end of the spinal cord, granular leucocytes containing pigment granules and fragments which closely resemble the fragments of degenerating nuclei. It may be that the leucocytes appear at this time and dispose of nuclear fragments. After one day, the degenerating nuclei are too rare to be significant.

The degenerative process which the foregoing facts seem to show, may be indicated diagrammatically as follows:

Cells directly cut → broken nuclei → rounded nuclei → fragments → disposed of by outbreak of cerebro-spinal fluid, or by leucocytes.

Cells just in front of those cut → angular nuclei → rounded nuclei → fragments → disposed of by leucocytes.

2. *Enlargement of Nuclei.*

A few preparations of the spinal cord soon after the operation show plainly that the nuclei near the end, but just in front of the deeply-staining nuclei, are larger than those of the normal cord. The long axes of nuclei close to the edge were measured and compared to nuclei of the same preparation which are some distance forward in the old tissue (Table I.). Immediately after the operation and in the very early regeneration stages, the nuclei near the end are larger, but the difference decreases until after nine and a half hours it is hardly significant. This enlargement might be preparatory to normal division or it might be a swelling which is a degenerative change preliminary to fragmentation Since this size difference is greatest at the very beginning and decreases during the first day until it is no longer significant, and since mitotic divisions are not seen in numbers until the third day, the enlargement is probably an early stage in nuclear degeneration.

Table I.

Time of Regeneration.	Nuclear Length Close to Edge.	Nuclear Length in Front of Edge.	Difference in Length.
Normal	7.9	7.5	.4
Immediately	10.5	7.8	2.7
1 hour	11.3	9.1	2.2
3 hours	8.6	7.3	1.3
5.5 hours	12.8	10.5	2.3
9.5 hours	8.1	8.0	.1
14 hours	7.6	8.3	−.7
1 day	8.2	6.6	1.6
2 days	8.1	8.0	.1
3 days	8.2	8.9	−.7
4 days	8.4	8.2	.2
6 days	10.7	10.4	.3

Explanation.—Each measurement recorded here is the average of the measurements of 9 or 10 nuclei. These were recorded in terms of the spaces of the ocular micrometer, but since one space was equal to approximately one micron (.955), the measurements were not transposed.

3. *Temporary Partial Closing of the Spinal Cord.*

After the degenerative process is complete and the deeply-staining nuclei have disappeared, the end of the nerve cord starts to close over. By the first day, the nuclei in the end of the cord have begun to pull apart, stretching out the connecting cytoplasm (Fig. 11). In general they extend toward the opposite wall of the central canal, thus narrowing the opening at the end. Some sections show pseudopod-like cytoplasmic extensions of the cells into the central canal as if closing were to be produced

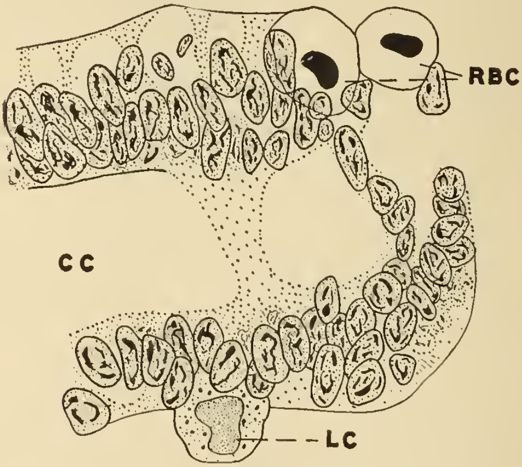

FIG. 9. Sagittal section close to the edge of the central canal, showing a row of cells, not quite at the end, extending across the central canal. Other sections of the series show that the end of the cord is still open. *cc*, central central canal; *rbc*, red blood corpuscles; *lc*, leucocyte. (920 diameters.)

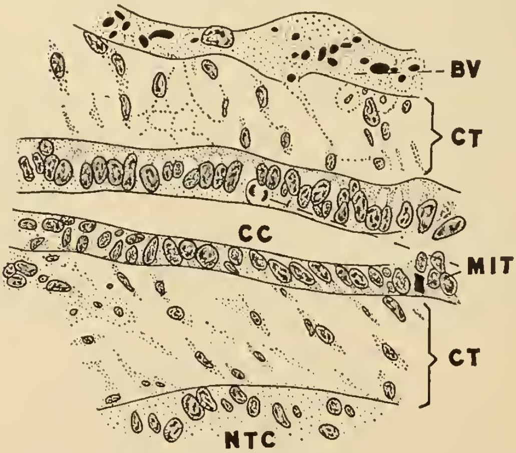

FIG. 10. Sagittal section through the new spinal cord six days after the operation. *bv*, blood vessel; *mit*, mitotic figures; *ntc*, notocord; *ct*, connective tissue. (330 diameters.)

by amoeboid movement of the cells. Figure 9 shows a section through one side of the cord, in which one layer of cells, not quite at the end, is extending down into the central canal. Up to about six days phenomena such as these may be seen, but sections from six to sixteen days show that the closing is not completed within that period. By sixteen days the new tail is almost as long as it will become (Durbin, 1909), and the spinal cord reaches back close to the epidermis at the posterior end. Still these later preparations show the sides of the neural tube gaping open, and red blood corpuscles extending forward into the central canal of the new cord, as if the pressure of the cerebrospinal fluid is not sufficient to keep them out.

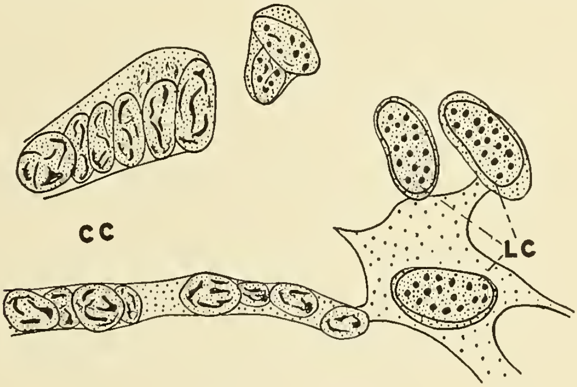

FIG. 11. Sagittal section through the spinal cord one day after the operation showing the granular leucocytes at the end of the cord, and the pulling apart of nuclei in the lower part of the cord. *cc*, central canal; *lc*, leucocytes. (920 diameters.)

4. *Cell Division.*

In an organ such as the spinal cord in which the nuclei lie close together, it is difficult to determine an amitotic division. In order to be sure that amitotic divisions do occur, one must find continuous stages in nuclear and cellular constriction without the formation of chromosomes. Because of the massing of nuclei, this cannot readily be determined in the normal spinal cord, though the slides were examined with this point in mind. The present study gives no evidence that normal nuclei divide amitotically, but stages in direct division can be seen in the deeply-staining nuclei at the cut edge. Is this amitosis or fragmentation? Do the daughter nuclei form normal nuclei, or do they divide several times and degenerate? There is no definite evidence that nuclei which divide directly ever become normal again. But at successive stages the deeply-staining nuclei become smaller and smaller down to fragments, so that the direct division is probably a fragmentation as a part of the degeneration of injured nuclei.

Mitotic divisions can easily be distinguished by the formation of chromosomes. All the preparations were examined and the distance of each mitotic division from the cut edge was measured. The results are shown in Table II. In the sections of the normal tail the number of divisions is the smallest, but since up to three days the mitoses are scattered and the number of individuals small, there is no reason for considering these mitoses anything but normal. During the period of degenerating nuclei, there are almost no mitotic divisions close to the edge. On the third day, the nuclei just in front of the cut edge are proliferating rapidly; at four days there are a few divisions past the cut; at six days there are almost as many divisions in the new spinal cord as in the old; at eight and nine days most of the divisions are in the new cord; at fourteen days there are scattered mitoses only, both in the old and new cord, and at sixteen days most of the dividing cells are in the very end of the new cord. If later divisions follow this general trend, it seems likely that the rest of the spinal cord will be formed by a growing zone at the tip, and until the new cord is complete the number of mitoses near the tip would probably decrease gradually.

Fig. 12 gives the average number of mitoses in the spinal cord at each stage, and therefore represents the rate of growth at these times. On the second day there is a considerable mass of tissue over the whole wound, though only degenerative changes have been taking place in the nerve cord. Beginning about this time, the nuclei in the end of the cord loosen and draw apart somewhat, stretching out the cytoplasm between them (Fig. 11). This is apparently the first extension in length of the spinal cord. At three days active proliferation of cells has begun but the pulling apart or stretching toward the cut edge continues. Fig.

Table II.

Regeneration Time.	No. of Individuals.	Mitoses in Old Cord.	Mitoses in New Cord.	2.8 mm.	2.1 mm.	1.4 mm.	.7 mm. ← Old Tissue.	New Tissue. →	.7 mm.	1.4 mm.
Normal . .	3	⅔	0		⅓	⅓		Position of Cut.		
Immed. . .	3	2⅔	0	⅓	⅓	⅓ ⅓ ⅔	⅓ ⅓			
1 hr.	3	6	0	⅔ ⅔	⅔ 1 ⅓	⅓ ⅓ ⅔	⅓ ⅓ ⅔			
3 hrs.	2	1½	0	1	½					
5½ hrs. . . .	2	2½	0	1 ½		½	½			
9½ hrs. . . .	2	1½	0		½ ½	½				
14 hrs. . . .	2	3½	0	½	½ ½	1 ½	½			
1 day	3	1⅓	0		⅓	⅓	⅓ ⅓.			
2 days. . .	4	1⅓	0		⅓	⅓	⅓ ⅓			
3 days. . .	1	19	0				6 4 8 1			
4 days. . .	2	15	2½	½ ½ 1	½	½	½ 3 2½ 6	1 1½		
6 days. . .	2	19½	19	½ ½ ½		1 1 ½ 2	2½ 2 2½ 4 2½	4 3½ 2½ 6½ 2½		
8 days. . .	1	2	40				1 1	3 12 6 5 4	1 3 6	
9 days. . .	1	16	42			1 1 1 3	3 1 6	2 7 4 5	3 3 4	9 5
14 days. .	2	6	6		½	½ ½ 1½	1½ 1 ½	1 1 ½ 1	½ 1 1	
16 days. .	2	½	7½			½		1 ½		3 3

This table represents the position and number of mitotic divisions taking place at different times. In most cases the number given is the average obtained from 2 to 4 individuals, as indicated in the table.

10 (six days' regeneration) shows the cells in one part of the cord stretched out to such an extent that vacuoles are left between the cells. It is during the period from four to sixteen days that most of the increase in length takes place, by active proliferation and migration of cells.

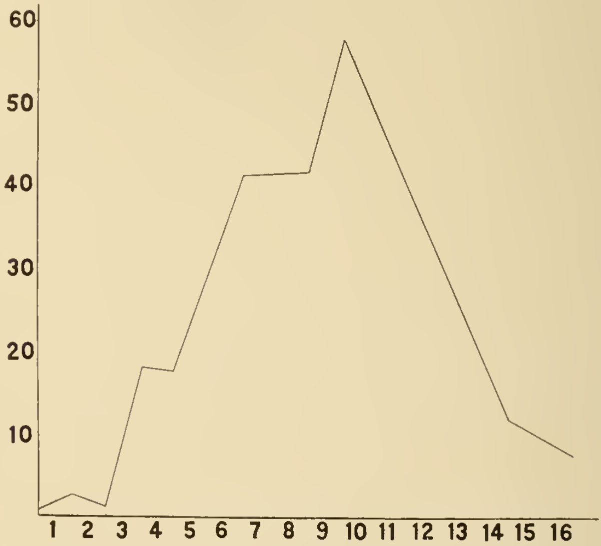

FIG. 12. Curve giving the number of mitotic divisions in that part of the spinal cord within 3 mm. of the edge. Beyond 3 mm. the mitoses are scattered. An abscissa represents the period of regeneration and the corresponding ordinate gives the average number of mitoses found in the individuals killed at the end of that period.

IV. DISCUSSION.

1. *Amitosis and Fragmentation.*

Fraisse in describing the regulative process at about two days after the operation, says; "Bereits früher machte ich darauf aufmerksam, dass am Wundrande eine starke Auswanderung von Leukocyten stattfindet, und dass diese es sind, welchen vor allen Dingen die Bildung des homogenen, lymphartigen Saumes, welcher zuerst die wunde bedeckt, zuzuschreiben ist. Das Rückenmark geht nun an meinen Schnitten bis dichte an diesen homogenen Saum heran, und die Elemente, welche es zusammensetzen, lassen sich immerhin noch nach 24 Stunden auch an diesem Saum von einander trennen, dann aber tritt eine bedeutende Wucherung von Kernen auf, und zwar scheint dieselbe auszugehen von den sogenannten Körnern,[1] deren Inhalt völlig homogen und stark lichtbrechend erscheint. Durch Picrocarmin werden diese Elemente ebenfalls stark tingirt, und nun sieht man an diesen nahezu gleich grossen Körnern Kerntheilungen, ohne dass jemals eine Spur von karyokinetischen Figuren constatirt werden konnte, in der Weise auftreten, das der Kern oder die Körner sich in der bekannten Weise schuhsohlenförmig einschnüren, und dass dann aus beiden Hälften Elemente gleicher Art hervorgehen. Nicht nur eine einmalige Einschnürung glaube ich beobachten zu können, sondern auch eine mehrfache, so dass der Kern sich bei diesem Process nicht nur in zwei, sondern auch in mehrere Stücke theilen kann."

Fraisse discusses further the evidence that the nuclei from the end of the spinal cord, which are found in the lymph-like border, divide amitotically. This agrees with the present observations. But he is satisfied to show that direct division does take place. So far as my preparations show, there are few evidences that the nuclei which divide amitotically afterward become normal nuclei. In some of the preparations of stages at which the deeply-staining nuclei have almost disappeared, there are a few nuclei which stain only slightly darker than the normal ones, and at this time there are no stages between these and the fragments. These few slightly darkened nuclei may, then, be forming normal nuclei again. All other evidence points towards the conclusion that at successive stages, these deeply-staining nuclei become smaller and smaller as if fragmentation or repeated direct division, is taking place. The conclusion from these facts is that nuclei which have only started to degenerate may perhaps return to the normal condition, but that nuclei that have gone so far as to divide amitotically are destined to fragment.

2. *The Appearance of Leucocytes.*

Barfurth (1891), working on the regenerating spinal cord of the frog larva at forty-six hours and at three days, makes the following statement: "Die unterste Theil des regenerirten Medullarrohres beherbergt in seinem Innern und zwischen seinen Epithelzellen zahlreiche fettig degenerirende Leukocyten; viele kleine und grosse Fetttropfen, die man hier überall findet, führe ich ihrem Ursprunge nach auf solche zerfallene Wanderzellen zurück. Ausserdem finden sich hier auch viele Pigmentkörnchen, die wohl bei der regressiven Metamorphose der zerfallenden Leukocyten entstehen (Pigmententartung)."

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[1] Körnern-nuclei of the gray substance, which are not present in the distal region of the spinal cord.

Barfurth figures the spinal cord of a larva of *Triton cristatus* after the sixth day of regeneration, in which these leucocytes and fat drops are shown. His figure is very similar to Fig. 11, which shows a section of a tadpole killed twenty-four hours after the operation. Both Fraisse and Barfurth mention particularly the presence of leucocytes in the early regeneration stages, but in the present study, leucocytes were not found in large numbers. Up to the end of the first day, none at all were seen close to the spinal cord. The earliest stage mentioned by Barfurth is that after a forty-six hour regeneration period, and this probably accounts for the different interpretation he gives of the origin of the "Fetttropfen" or fragments. If these fragments are followed back into earlier stages in my sections, they become larger and larger and are seen to be identical with the degenerating nuclei. To be sure, the leucocytes when they first appear in the spinal cord region contain what might be called fat drops, but is it not more reasonable to suppose that the leucocytes which are present at this time dispose of the fragments of injured spinal cord nuclei?

3. *Temporary Closing of the Spinal Cord.*

Barfurth describes the closing of the spinal cord at three days by means of cytoplasmic extensions of the cells, such as were seen in the preparations used in the present study. "Der sich wieder ansammelnde Liquor cerebrospinalis drückt nun auf die neugebildeten, noch wenig resistenten untern und seitlichen Theile des Rohres, und treibt sie kolbenartig auseinander. Die Zellen passen sich einstweilen durch ihre Lagerung diesem Druck an und behalten später diese Lage noch eine Zeit lang bei." Barfurth mentions this as a temporary closure of the spinal cord, so his later preparations evidently show the cord again open. The regenerated spinal cord at sixteen days has almost reached its maximum length, but it is not yet closed. Whether or not the completely regenerated spinal cord is open at the end or closed as in the normal tail cannot be answered by the present study.

4. *Rate of Division. Amitosis versus Mitosis.*

Durbin (1909), in analyzing the rate of increase in length throughout the regenerative process in the tail of *Rana clamitans*, distinguishes four periods. "The operation was followed by an interval of low rate, succeeded by one of rapidly increasing rate, then by one of rapidly decreasing rate and finally an interval in which the rate gradually approaches zero. The first low period is explained by a combination of two factors, (*a*) the shock of the injury, and (*b*) the formation of a cap of embryonic cells which is to serve as a basis for the more active regeneration. The second or period of rapidly increasing growth is the one in which practically all the cells in the new part are undifferentiated and rapidly dividing. The third and fourth periods are explained by the appearance of differentiation, which lessens the number of dividing cells."

Fig. 12, based on the number of mitotic divisions in the spinal cord, shows these same periods. The initial period of low rate covers the first two days; that of rapidly increasing rate includes the third to ninth days; the period of rapidly decreasing rate extends from the tenth to sixteenth days, and the period of gradually decreasing rate, though not covered in the present work, would undoubtedly extend on from about sixteen days. In the light of this histological study, a somewhat different interpretation might be given to the initial period. It is during these first two days that degeneration of the injured cells is taking place. Though at this time a cap of undifferentiated cells is being formed over the wound, the spinal cord does not participate in the formation of this cap, nor is any such cap formed at the end of the spinal cord. Since the spinal cord cells in this part of the tail are so slightly differentiated, the new cord is formed from the old without the separation of a group of special embryonic cells.

The similarity of the rate curves based on a counting of the mitotic divisions with that based on the amount of tissue formed at each period, seems to be significant. It shows that the rate of tissue formation is closely correlated with the number of mitotic divisions. Considering amitosis, this may be interpreted in one of two ways—(1) either the number of amitotic divisions is similarly correlated with the rate of growth so that the total number of divisions both mitotic and amitotic, gives the same form of curve as the mitotic divisions alone, or else (2) amitotic divisions are not numerous enough to be significant. The former explanation is improbable. The nuclear conditions producing mitotic division are probably different from those producing amitotic division. Different cells in the same region may divide by different methods, but it is very improbable that the conditions producing one form of division would increase and decrease in influence at the same rate and the same times as those producing the other form. Moreover, in the present study, no examples of direct division were seen except in the degenerating, fragmenting nuclei. This similarity of the rate curve of mitotic divisions to the rate curve of growth is evidence, other than the negative observational evidence, supporting the view that amitotic division is not important in the formation of this organ by regeneration.

V. Summary.

1. The regenerating spinal cord of the frog tadpole has been studied histologically in order to learn the mechanism, or the stages in the process, by which the new cord is formed from the old.

2. During the first day after the operation, injured nuclei in the end of the spinal cord degenerate. There is first a decrease in size, by contraction or loss of achromatin, and then a fragmentation of these degenerating nuclei. The fragments may be carried away either by the outbreaking of a cerebrospinal fluid or by leucocytes which appear at this time. These fragments are parts of disintegrated spinal cord nuclei and not of leucocytes.

3. From the second to the sixth days there is a temporary partial closing of the neural tube, probably by migration of the cells near the end.

4. The new cord is formed by the cells of the old cord near the cut edge, by mitotic division and migration.

5. The number of mitotic divisions at different periods is proportional to the rate of regeneration at those periods as determined by Durbin. Amitotic division, if it occurs, is not important in the formation of the regenerated organ.

6. There is no observational evidence from this study that amitotic division does occur in normal regenerating spinal cord cells.

This work was carried on under the direction of Dr. Charles Zeleny. His suggestion of the problem, and constant interest in its progress are sincerely appreciated.

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ABNORMALITIES AND REGENERATION IN CICINDELA.*

By Victor E. Shelford.

I. Introduction.

The occurrence of certain abnormalities of the elytra and labrum of Cicindela collected in the wild state and in adults reared in the laboratory and the almost entire absence of abnormalities of the legs and antennæ led me to bring together the main facts at my disposal and to perform a few experiments to determine if possible the cause of the abnormalities. It is the purpose of this paper to point out the possibility of the use of the material for studies of the physiology of melanin pigment distribution by operative experiments during development.

II. Types of Abnormalities.

The abnormalities noted are confined chiefly to the elytra and the labrum. Figure 1 shows an abnormal labrum of a specimen of Cicindela tranquebarica of the green Nevada type with the small spots on the margin of the elytron as the only vestige of the usual markings of the species (Wickham '06). This labrum has a slender smooth and shiny projection at one side but suggesting a tearing from the center toward the outside which resulted in the slender strip being left hanging down freely. Such abnormalities are not common.

A second and relatively common type is shown (figures 4 and 5) in which one elytron is longer than the other and the form and color pattern sometimes modified. No abnormal legs have been noted in collected specimens.

III. The Production of Abnormalities in Experiments.

To learn something of the abnormalities of the labrum and legs twenty-six late larvæ and six pupæ of Cicindela punctulata were operated on. The operations were performed with sterile scissors and the larvæ placed in vertical burrows in sterile soil kept moist with a hydrogen peroxide solution. The operations were confined to the labrum and right hind leg.

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* Contribution from the Zoological Laboratory of the University of Illinois. No. 46.

Four larvæ were cut in the center of the labrum; six pupæ were operated upon in the same manner. All the pupæ were treated essentially alike being cut from the center of the pupal labrum toward the left side and slightly upward. The results are tabulated below:

Location of cut	Part Removed or Attached	No. Operated on	No. Surviving	Results in Adult
In leg, Tarsal joints	removed	4	2	One showed shorter tarsal joints.
In leg, Tarsal joints	attached	4	3	No modification of adult.
Mid tibia	removed	9	4	No modification of adult.
Mid tibia	attached	2	1	No modification of adult.
Tibia femur joint	removed	3	2	No modification of adult.
Larval labrum in center	attached	4	4	No modification of adult.
Pupal labrum	attached	6	6	4 showed modification 1 recovered completely

The result of removing the legs are all the same except for the one shown in figure 8 which is a little shorter, has a little shorter spur and less numerous and shorter hairs. The pupal legs evidently develop in the upper part of the larval leg and modification results only when the basal portion is injured. Megusar '03, obtained similar results with other beetles. This fact explains the rareness of leg abnormalities.

The experiments on larval labrums gave no results. The pupæ operated on only emerged as adults in three cases. One died before the cuticula hardened. This individual's labrum had completely healed but a very dark area occupied the area near the cut. One that failed to emerge had healed the wound completely. The labrum of the other had almost degenerated. The two seen in figures 2 and 3 show some similarity to the one collected in Nevada. The Nevada specimen suggests that the abnormality is due to a tear in the labrum probably at the time of the last larval moult. The larval cuticula often sticks to the anterior part of the pupa when the surrounding conditions are dry and the Nevada dry climate would favor such accidents.

Elytral abnormalities shown in two species collected in Kansas were duplicated in Cicindela limbalis reared from a larva. Here a reduced color pattern accompanies a short elytron as in the specimen of wild tranquebarica. While this is the only elytral modification of exactly this type noted in wild individuals a type with holes in the elytra is more common in experiments. For example, two holes completely healed occured in an elytron with an irregular and distorted pattern, Fig. 7. While several hundred larvæ of each of several species were reared to maturity this kind of abnormality occurred only three or four times.

The peculiar elytral modifications are probably due to rough handling. It seems probable that the abnormal conditions result from pressure or slight injury during the pupal stage. The elytron being much crowded, the folds projecting outward are particularly liable to injury. The elytral openings in Fig. 7 are rounded and smoothly healed, the wing cavity being entirely closed and with cuticular covering on the edges. The comparatively frequent occurrence of elytral modifications in reared specimens as compared with their rather rare occurrence in nature justifies the above assumption. The abnormalities are not unlike those of Drosophila described by Morgan as mutations. Since the tiger beetle abnormalities occur in animals reared from wild stock which show extremely few such variations in thousands of specimens collected in the wild state an unusual burden of proof is necessary to establish such conditions as anything but abnormalities produced again and again by the necessary rough handling of cultures. If such proved true their apparent inheritance in Drosophila may only be a sensitiveness to handling.

The abnormalities of patterns and the reproduction of black pigment in wounded labral surfaces indicate that such material in the hands of a skillful investigator might, with suitable operations, show something of the physiology of pattern production. If the distribution of pigment can be controlled by suitable operations as is indicated by the work, it will have important bearing on the studies of insect patterns.

University of Illinois, May 19, 1915.

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EXPLANATION OF PLATE XXIV.

Fig. 1. Abnormal labrum of a blue Nevada form of C. tranquebarica.

Figs. 2 and 3. Abnormalities in C. punctata produced by cutting into the labrum during the pupal stage.

Fig. 4. Long and short-elytroned form of C. sexguttata from Topeka, Kansas.

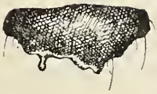
Fig. 5. Specimen of C. tranquebarica with short elytron bearing an abnormal pattern, from Dodge City, Kansas.

Fig. 6. Reared specimen of C. pupurea limbalis a right elytron similar to that of tranquebarica (Fig. 5). Note reduced markings. This deformity is supposedly due to rough handling.

Fig. 7. Elytron of C. limbalis with healed openings due to the death of certain parts of the folded pupal elytron.

Fig. 8. Right short hind leg of a specimen of C. punctulata in which the tarsal joints were removed just before pupation. The hairs are not well developed.

Fig. 9. Left normal hind leg of the same specimen of C. punctulata.



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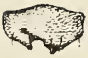

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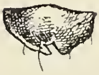

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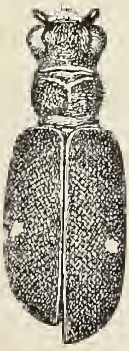

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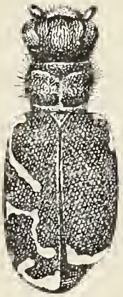

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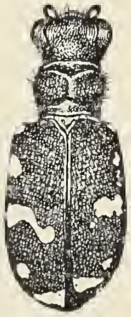

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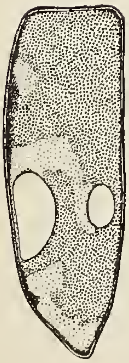

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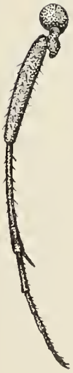

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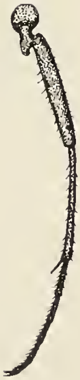

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V. E. Shelford.

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Three New Gregarines from Marine Crustacea

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Oyster Bay, Long Island, N. Y.

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THREE NEW GREGARINES FROM MARINE CRUSTACEA

MINNIE E. WATSON
Oyster Bay, Long Island, N. Y.

THREE NEW GREGARINES FROM MARINE CRUSTACEA *

Minnie E. Watson
Oyster Bay, Long Island, N. Y.

The protozoan parasites described here were studied during the summers of 1913 and 1914 at the Biological Laboratory of the Brooklyn Institute, Cold Spring Harbor, L. I., and I wish to thank Dr. C. B. Davenport, the director, for kindly affording me the privileges of the laboratory.

All the hosts were found on the Sand Spit, a narrow peninsula half a mile long separating the outer and inner harbors. The area is thus geographically very restricted, and although the hosts are similar and the possibility of all of them being infected with the same parasite considered, yet there is abundant evidence to warrant regarding the three forms described below as separate species.

Frenzelina delphinia nov. spec.

Figures 1 to 8

The host of this gregarine is the large white sand flea, *Talorchestia longicornis,* which is found in fine sand between tide marks. Fleas were taken from the eastern end of the Sand Spit, and also from locations along the road to Lloyd's Neck, from Huntington Beach, and from Northport Harbor; and hosts from all these localities were found to be infected.

The parasites were present in 30 per cent. of the 260 intestines examined, the number in an infection varying from one to ten in 75 per cent. of the hosts and from ten to 500 in the other 25 per cent.

The sporonts (Figs. 1 and 2[1]), which live free in the lumen of the upper part of the intestines, are small, the average of fifty large individuals being 110μ in length and 60μ in width. The largest individual seen measured 115μ in length and 64μ in width. The ratio of length of protomerite to total length of sporont is about 1 : 4; the ratio of width of protomerite to width of deutomerite about 1 : 1.5. A table of a few measurements is given at the end of this section.

The sporonts are biassociative when mature and the two members of an association do not differ materially in size; either the primite or the satellite may be a little longer or broader than the other. The sporonts are stout bodied, being less than twice as long as wide. The protomerite is cylindrical and broadly rounded in front and is about three fourths as high as wide. There is often a slight constriction at the septum which separates the protomerite and deutomerite. The deutomerite is doliform, two thirds as wide as long, widest through the middle portion, and terminates in a broadly rounded extremity. There is a small conspicuous papilla at the anterior end of the protomerite of the young sporonts which persists, although reduced in size, in the adults. In the satellite of an association it becomes a structure for the attachment of the two sporonts by projecting upward and forming a small indentation in the posterior end of the primite (Fig. 4). Hydrochloric acid, either dilute or concentrated, fails to dislodge the two sporonts, but sodium hydroxid or ammonium hydroxid, in solution, readily disassociates them.

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* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 60. Also from the Biological Laboratory, Cold Spring Harbor.

[1] For explanation of special terms used see description of Figure 1.

The endocyte of the mature sporonts is light brown in color and in full-grown animals fairly dense, and the nucleus not visible. Young sporonts are less dense and the color paler. The satellite of an association is generally less dense than the primite, and for this reason its nucleus is often visible in vivo when that of the dense primite is not. The protomerite of a sporont is slightly less dense than the deutomerite and contains fewer protoplasmic granules; the granules are slightly larger than those in the deutomerite and the endocyte paler in color. When the host has just been taken and has probably fed the night before, full grown sporonts are dense; when, however, the fleas have been kept in damp sand for a few days without food, the parasites are likewise deprived of nourishment and become pale in color and the number of protoplasmic granules present, hence the density, is greatly reduced. The addition of a drop of iodin solution renders these pale parasites visible but of course kills them.

The nucleus of the sporonts is generally visible in vivo only as a lighter indefinite area although in young and starved sporonts it is often visible. It is large and spherical and contains one or two large homogeneous karyosomes. The epicyte is thin and fragile and is marked with longitudinal striations only visible under an oil immersion lens.

Cross sections made of the intestine of the host reveal the fact that development is intercellular as in the family of the Stenophoridae. This is the first genus of the family Gregarinidae which has been found to develop in this manner, the other members possessing an epimerite which alone is attached to the host cell, the remainder of the trophozoite projecting into the lumen of the intestine. In the species here described, the whole trophozoite is embedded in the epithelium (Figs. 3 and 6). When stained with Delafield's hematoxylin, the cells of the epithelium become purplish-blue, while the parasites stain less deeply and the color is a clear homogeneous blue with a darker blue nucleus.

The animals are capable of movements both of bending and of gliding progression. The sporonts are able to move through a narrow place in the manner employed by an amoeba. When the host intestine is first opened, a mosaic of inert distorted individuals or associations lying near the epithelium is often revealed; when, however, water or normal salt solution is added, the smooth, regular contour is quickly restored. Normal salt solution stimulates movement and sporonts often remain alive and motile for an hour and a half. Sea water has the same effect. As motion tends to be retarded at the end of this time, weak tannic acid solution was added in a few instances and caused considerable acceleration of movement; plasmolysis, however, occurred inside of another half hour and the animals died.

That transparent threads of mucus are present at the posterior end of the body was frequently attested by the fact that the animals were able to carry with them in their movements large or small masses of débris at a distance behind the body often as great as the length of the animal itself. A mass twenty-five times the volume of the gregarine itself was in one instance observed being carried along.

Cyst formation was observed in material from the host intestine from its incipiency until rotation ceased. The adult sporonts free in the intestine which are ready for cyst formation become thickened and shortened, motion becomes sluggish, movement of progression ceases and that of bending becomes more active (Fig. 5). Concomitant with the revolving motion there occurs a deposition of gelatinous threads exuded from the body in fine concentric layers around the revolving mass. The sporonts become a spherical mass and the threads form a thick cyst wall. The rotating mass passes from the mid intestine to the rectum and ceases motion (Fig. 7). It begins here its development by the loss of the wall separating the two sporonts and the disintegration of the two sporont nuclei. The protoplasm of the cyst collects in masses and on the periphery of each protoplasmic mass are formed spherical protuberances, the gametids. The cyst is now expelled with the feces. Cysts when expelled are somewhat opaque, tan in color, and average 80μ in diameter, including the cyst wall.

It is difficult to effect cyst development to completion by artificial methods. Marine bacteria seem to be virulent and to cause putrefaction or otherwise stop development in the early stages. Cysts were kept in the damp chamber in normal salt solution and a few yielded gametes upon being crushed when 24 hours old. The gametes were stained with safranin; they were large and nearly spherical, and no difference in size was observed in those from the same cyst. Some of the gametes, however, showed large deeply staining nuclei and others smaller nuclei which stained less deeply, probably because of their reduced chromatin content. A difference in the staining reaction was also noted in the two sporonts in a cyst of about eighteen hours, the one sporont mass staining more deeply than the other.

A very few cysts developed to completion and dehisced by simple rupture in 35 hours, but no well formed spores were present.

Orchestia agilis, the smaller very common sand flea, was found in one instance to be infected with three nonassociative sporonts which agree in size and shape with the species described above. This flea was also heavily parasitized with an infusorian. *Orchestia grillus* from the roots of the eel grass (*Juncus palustris*) was not found to be infected with gregarines.

The genus Frenzelina (Léger and Duboscq, 1909) has not hitherto been reported from the United States and only seven species have been described. The species described above is placed in this genus because (1) the sporonts are biassociative, (2) the cysts dehisce by simple rupture, (3) development is intercellular, (4) the apex of the protomerite is slightly papillated, (5) the parasite inhabits the intestine of a crustacean.

I wish to designate the species, of which no previous record is found, *Frenzelina delphinia.*

The fact that development is intercellular was not determined by Léger and Duboscq and this important fact should be added to the features which characterize the genus Frenzelina.

A table of typical measurements, in microns, follows:

Total length association	215	215	210
Primite:			
Total length sporont	115	110	112
Length protomerite	20	24	27
Length deutomerite	95	86	93
Width protomerite	39	35	43
Width deutomerite	62	66	63
Ratio length protomerite: length deutomerite	1 :4.7	1 :4.6	1 :3.5
Ratio width protomerite: width deutomerite	1 :1.6	1 :1.8	1 :1.5
Satellite:			
Total length sporont	100	105	98
Length protomerite	20	20	20
Length deutomerite	80	85	78
Width protomerite	37	40	40
Width deutomorite	55	56	60
Ratio length protomerite: length deutomerite	1 :1.5	1 :1.4	1 :1.5
Ratio width protomerite: width deutomerite	1 :1.5	1 :1.4	1 :1.5
Cyst, outer diameter	77	90	86
Inner diameter	63	74	66
Thickness transparent layer around cyst	7	8	10

Frenzelina olivia nov. spec.

Figures 8 to 10

The host of this species is the small littoral spider crab, *Libinia dubia,* which is abundant along the shores of Long Island Sound and its inlets. The parasite is found in the upper part of the intestine. It generally occurs in moderate numbers (10 to 100) but rarely an infection of 1,000 or more is encountered.

The sporonts are biassociative and average 80μ in length and 35μ in width. They are ellipsoidal in shape, rounded in front and rather blunt posteriorly. The protomerite is hemispherical, very slightly constricted at the septum. It is about one fifth the total length of the sporont and slightly papillate in the adults (Fig. 8); the younger solitary sporonts possess a conspicuous papilla (Fig. 10). The deutomerite is but little wider than the protomerite (1:1.2); in solitary individuals it is more broadly rounded at the posterior end than in those which are attached. The endocyte of the mature sporonts is dark brown and very dense in the deutomerite; the protomerite is less dense and tan in color. At the anterior end of the protomerite is an orange colored disc. The nucleus is visible only in immature specimens. It is spherical and generally contains one large karyosome.

Movement of progression is rapid and continues only for intervals of about two seconds.

Cysts are spherical, dark brown in color, and from 45 to 60μ in diameter including the enveloping wall. They occur in the posterior third of the intestine. No sections were made of the host intestine.

This species is placed in the genus Frenzelina because (1) it is biassociative, (2) there is a papillated conspicuously differentiated apical area in the protomerite, (3) it is very similar to *Frenzelina delphinia* in form and location and both occur in hosts from the same habitat; (4) it is parasitic in the intestine of a marine crustacean.

The larger spider crab, *Libinia emarginata,* which is found in deeper water and seldom comes near the shore, has been examined repeatedly for gregarines, but none have been found to date. Other crabs procured from oyster boats, and which were dredged in the Sound and Harbor, have not yielded gregarines. These include *Neopanope texana sayi, Carcinides maenas, Pagurus bernhardus* and *Pagurus longicarpus;* and *Chloridella empusa* from the mud flats of the Inner Harbor. From the south side of the island, the following crabs have been examined and none found to be infected with gregarines: *Emerita talpoida, Callinectes sapidus, Ovalipes ocellatus, Ocypoda albicans,* and an undetermined species of Orchestia.

It seems possible that only littoral marine crustacea are infested with gregarines and that spores are eaten along with shore vegetation, grasses and tide water algae, and are swept away by the tides or are noninfective when they reach the water.

A table of measurements, in microns, follows:

Total length association	218	195	150	127
Primite:				
Length sporont	100	85	80	65
Length protomerite	20	20	14	14
Length deutomerite	80	65	66	51
Width protomerite	35	38	30	30
Width deutomerite	43	48	45	36
Ratio length protomerite: total length sporont	1:5	1:4.2	1:5.7	1:4.6
Ratio width protomerite: width deutomerite	1:1.2	1:1.3	1:1.5	1:1.2
Satellite:				
Length sporont	118	110	70	62
Length protomerite	25	14	10	10
Length deutomerite	83	96	60	52
Width protomerite	36	39	22	22
Width deutomerite	36	50	28	30
Ratio length protomerite: total length sporont	1:5	1:8	1:7	1:6.2
Ratio width protomerite: width deutomerite	1:1	1:1.3	1:1.3	1:1.4

Frenzelina nigrofusca nov. spec.

Figures 11 to 14

Two species of fiddler crabs, *Uca pugnax* and *U. pugilator, which* live together at the roots of the eel grass, were found to be infected with a species of gregarine. About 30 per cent. of the hundred or more crabs examined were parasitized and the infection was very moderate; in only rare instances was the number of parasites present greater than fifteen.

The sporonts were solitary, none being associative as is characteristic of this genus. The body is broadly ovoidal, less than twice as long as wide (Fig. 11) and is often nearly rectangular in shape with rounded corners (Fig. 12). Sporonts average 100μ in length and 65μ in width. The protomerite is hemispherical and very slightly constricted at the septum; it is about one third the length of the whole sporont. There is a minute papilla at the anterior end; this papilla is large and conspicuous in the trophozoite (Fig. 13). The deutomerite is of approximately the same width as the protomerite and twice as long. It is very broadly rounded or often flattened posteriorly.

The endocyte is very dense and appears dark brown or black in transmitted light. It is but little less dense in the protomerite than in the deutomerite and in starved animals becomes tan in color. The nucleus is not visible in the live sporonts. The sarcocyte is relatively thick, especially over the anterior end of the protomerite. The nucleus is small and spherical and contains one or two minute karyosomes. Uniform gliding movement was observed at a relatively slow rate. The cysts are very dense and are spherical. Spores were not seen.

This species is placed in the genus Frenzelina for two reasons: (1) the protomerite possesses at its apex a small papillated and conspicuously colored disk, this papilla being well developed in the trophozoite, (2) the gregarine infects the intestine of a marine crustacean. While no associative sporonts were seen, the species is very probably associative from its affinities.

A table of measurements of a few specimens is appended here, all dimensions being given in microns:

Total length sporont..........	72	82	100	120	125
Length protomerite	20	22	22	31	31
Length deutomerite	52	60	78	89	94
Width protomerite	39	40	70	50	75
Width deutomerite	37	40	75	75	65
Ratio length protomerite: total length	1:3.6	1:3.9	1:4.5	1:4	1:4
Ratio width protomerite: width deutomerite	1:1	1:1	1:1	1:1.5	1:2.1
Diameter nucleus	1	1.1	1.5		

SUMMARY

1. Three new Gregarine parasites of the genus *Frenzelina* are described from marine Crustacea.

2. Parasites belonging to this genus have not hitherto been reported from the United States.

3. A new definitive character for this genus has been determined upon sectioning the host intestine, namely, the fact that the parasites are intercellular.

REFERENCE CITED

Léger, L., and Duboscq, O. 1909. Etudes sur la sexualité chez les Grégarines. Arch. Prot., 17: 19-134.

EXPLANATION OF PLATE

Frenzelina delphinia nov. spec.

1. An association of two sporonts from lumen of intestine of *Talorchestia longicornis; a,* protomerite of sporont, *b,* deutomerite, *c,* primite, *d,* satellite.

2. Another association with satellite much younger than primite.

3. Cross section of portion of intestine of *Talorchestia longicornis* showing intercellular development of parasite; *a,* oblique section of trophozoite embedded in epithelium; *b, c,* sections of sporonts lying free in lumen but near the walls and surrounded by mucus.

4. Interlocking device by which satellite of association is attached to primite.

5. Association revolving in an early stage of cyst formation.

6. Cross section of portion of host intestinal epithelium showing embedded trophozoite in longitudinal section.

7. Cyst soon after completion, showing transparent cyst wall and two sporonts still distinctly outlined.

Frenzelina olivia nov. spec.

8. Mature association from lumen of intestine of *Libinia dubia.*

9. Immature sporont from same location.

10. Young sporont free in intestinal lumen, showing papilla at anterior end of protomerite. Magnification greater than in Figures 8 and 9.

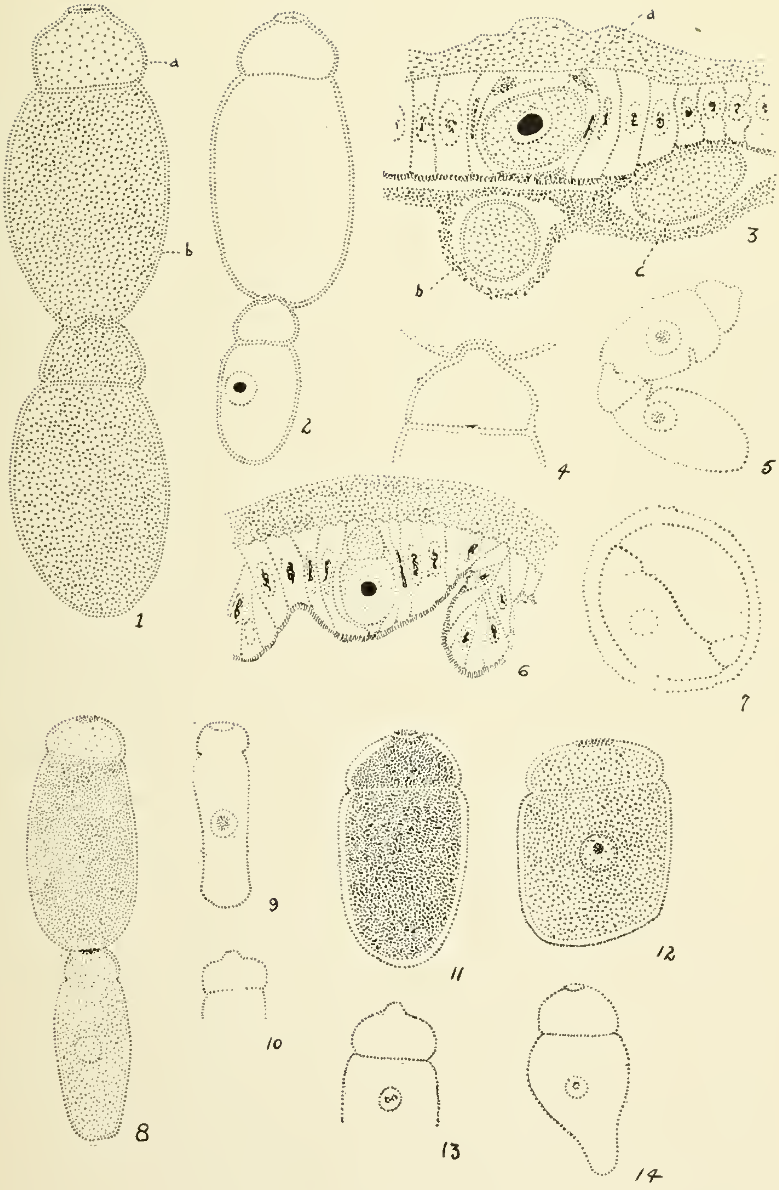
Frenzelina nigrofusca nov. spec.

11, 12. Adult sporonts from lumen of intestine of *Uca pugnax.*

13. Trophozoite from lumen of host, showing papilla.

14. Sporonts with posterior half of body contracted, indicating that considerable movement of the body is possible.

PLATE



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A New Infusorian Parasite in Sand Fleas

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A New Infusorian Parasite in Sand Fleas

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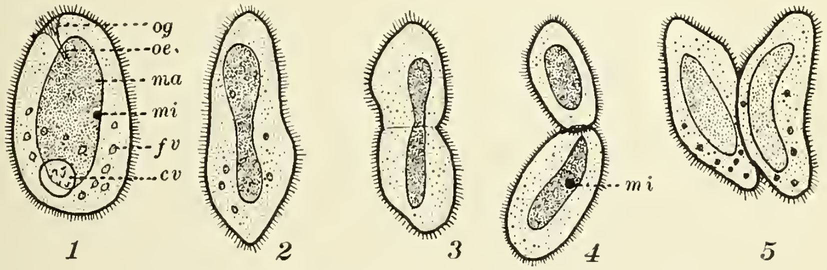
A NEW INFUSORIAN PARASITE IN SAND FLEAS *

Minnie E. Watson
Oyster Bay, Long Island, N. Y.

While examining some sand fleas on the shores of Long Island Sound for gregarine parasites, the writer found two hosts infected with an infusorian parasite; one of the hosts being the common small flea, *Orchestia agilis,* the other the larger *Talorchestia longicornis.* The two infected hosts were found in the same habitat and on the same day. The infusoria were found in only these two instances out of about 300 fleas examined for gregarines. The parasites infect the alimentary tract of the host in great numbers, several hundred being present in each flea.

In the vegetative stage, the animals are broadly ovoidal, tapering slightly at one end (Fig. 1). The macronucleus is very large, and ellipsoidal in shape; it is granular and homogeneous and does not stain deeply with Ehrlich's hematoxylin and acetocarmin. The micronucleus is small and deep staining, and lies contiguous to the macronucleus. The apical or subapical oral groove is small and inconspicuous; it leads to a short slender esophagus. Small contractile vacuoles were observed, not more than one being seen in a single individual. Many small food vacuoles were often present. Cilia of uniform length



Explanation of Figure

1. *Balantidium orchestium,* vegetative individual. *Og,* oral groove; *oe,* esophagus; *ma,* macronucleus; *mi,* micronucleus; *fv,* food vacuole; *cv,* contractile vacuole.

2, 3, 4. Three stages in binary fission.

5. Conjugation.

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* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 61. Also from the Biological Laboratory, Cold Spring Harbor.

cover the body, those in the oral groove being slightly the longer. The size of vegetative forms ranges from 300 to 360 microns by 180 to 220 microns.

In individuals which are ready for asexual reproduction, the body becomes elongate and tapers at the ends; the macronucleus becomes longer and constricted centrally, at the same time contracting in volume. It also stains more deeply than does that of the vegetative animal (Figs. 2, 3, and 4). When transverse fission is complete, the two nuclei attain their normal vegetative form and density. Conjugation was noted in one instance.

The parasite may be classified as follows:

1. Order Heterotricha: zone of large cilia leading to mouth.
2. Suborder Polytricha: body covered with an even coat of cilia.
3. Family Bursaridae: peristome broad, body broad and large.
4. Genus Balantidium: parasitic, inhabiting the alimentary tract of the host, body ovoidal or ellipsoidal, blunt at ends in vegetative stage, macronucleus ellipsoidal.
5. The parasite is closely allied to *B. coli* Stein and *B. elongatum* Stein; it differs from them in size of the body and in relative size of the nucleus. No previous record of this form has been found.

I wish therefore to designate this species *Balantidium orchestium.*

Urbana, Ill., June, 1915.

REFERENCES CITED

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